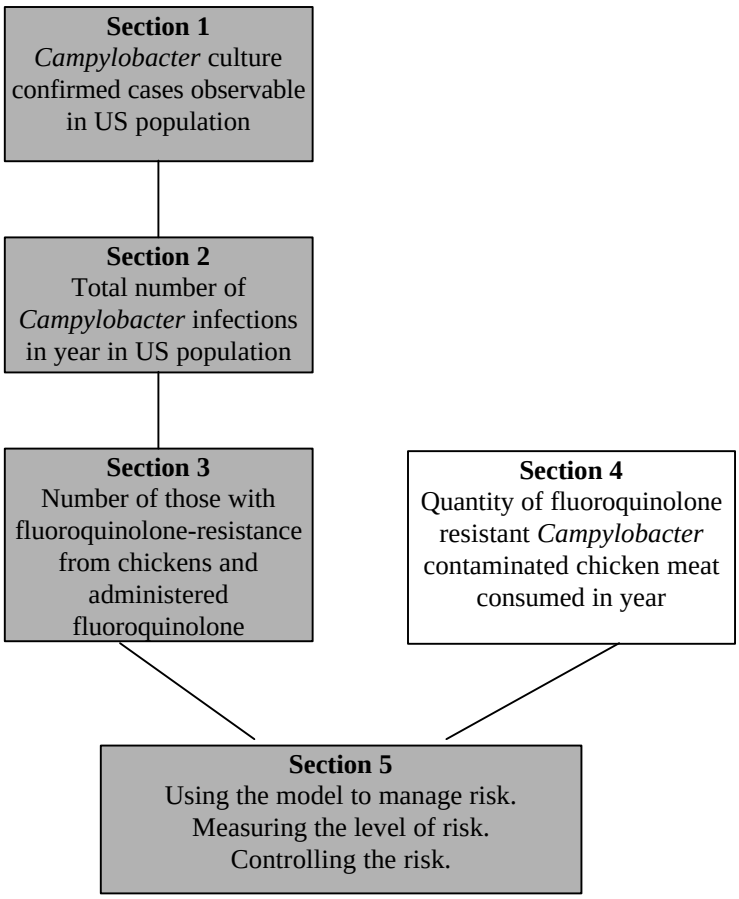
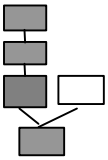
Section 4

Estimating year's consumption of domestically reared chickens contaminated with fluoroquinolone resistant *Campylobacter* in the US





Symbol	Description	Formula
p_c	Prevalence of *Campylobacter* in chicken carcasses at end of slaughter processing	Beta distribution based on data
p_{rc}	Prevalence of FQ resistant *Campylobacter* among *Campylobacter* isolates	Beta distribution based on data
p_p	Estimated prevalence of fluoroquinolone-resistant *Campylobacter* in broiler carcasses	$= p_c * p_{rc}$
c	Consumption of boneless domestically reared chickens in US per head (lbs)	Data
V_c	Volume of boneless domestically reared chicken consumed by US citizens (lbs)	$= c * n_{US}$
$\mathbf{V_i}$	Total consumption of boneless domestically reared chicken contaminated with fluoroquinolone resistant *Campylobacter* in US (lbs)	$=V_c*p_p$

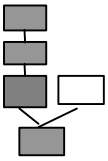
Overview for Section 4

This section estimates the burden of Ciprofloxacin resistant *Campylobacter* on chicken carcasses by multiplying the carcass *Campylobacter* prevalence by the level of resistance in isolates from chickens. An estimate of the proportion of domestically reared chicken with fluoroquinolone resistant *Campylobacter* using food disappearance data, less imports, was calculated to account for changes in chicken consumption from year to year.

Parameter estimations

4.1 (p_c) - Prevalence of *Campylobacter* in chicken carcasses at end of slaughter processing

Approximately 200 broiler slaughter establishments were included in the sample, representing 87% of all broiler slaughter establishments under Federal inspection in 1994. The broilers slaughtered at these establishments accounted for more than 99.9% of all broilers slaughtered during the period. Sample size, to provide reasonable levels of precision for a national prevalence, was estimated at 1200 samples. To achieve this number of samples a random number of 1871 broiler carcass samples were requested during the 52-week sampling period. Some samples were not collected, some were collected but not analyzed and the total number of samples providing laboratory results for the prevalence estimate was 1297 samples (81). Sampling frame was based upon weekly identification of randomly selected establishments using probabilities for sample selection that were proportional to the slaughter volume of the selected establishments, therefore those establishments slaughtering a greater number of chickens were sampled more frequently than other establishments. Sample delivery constraints resulted in the restriction of sampling to first shifts, Monday through Thursday. Carcasses were obtained from the drip line after the chill tank, the end point for slaughter and evisceration and prior to further handling and processing. Whole carcasses were randomly selected, and aseptically placed into a sterile bag that was securely closed, double bagged, packed with a gel pack and shipped to the laboratory via overnight delivery service. Only samples received at temperatures between 0 to 10 degrees C (inclusive) within one day of sample collection were analyzed. The analytical sample was obtained from rinse fluid recovered after shaking the broiler carcass in 400 ml of sterile Butterfield's Phosphate Diluent (81). Isolation was achieved using Hunt's Enrichment Broth, incubating the sample for 24 hours in a microaerophillic environment (5% O_2, 10% CO_2 and 85% N_2), followed by streaking onto Modified *Campylobacter* Charcoal Differential Agar for isolation of *Campylobacter* spp after incubation at 42 degrees C for 24 hours (62). Tests to identify *Campylobacter jejuni* and *coli* included wet mount examination, glucose fermentation, catalase, nalidixic acid, and oxidase tests. Nalidixic acid screening was performed to eliminate *Campylobacter* spp other than *jejuni* and *coli* from the prevalence estimate. Since fluoroquinolones were not licensed for use in poultry during the survey period, it was assumed that the level of nalidixic acid resistant isolates was low



in 1994-5. Therefore, the prevalence estimate was unlikely to be affected by acquired resistance and potential misclassification of Campylobacter species.

ASSUMPTION: If a carcass was positive for *Campylobacter*, the predominant species isolated was *C. jejuni*.

The *Camplyobacter* prevalence estimate from the drip line was preferred because at this point carcasses were ready for further processing and had the least potential of human or other non-chicken sources of contamination. Post-chiller sampling of carcasses takes into account the cross-contamination from other chickens that occurs while in the chiller that leads to carriage of many diverse strains of Campylobacter on a single chicken product (71). The post-chiller location is a sampling point that is repeatable, practical, and provides isolates for susceptibility testing, closely linking these two parameters to provide a better estimate of the level of resistance. This would be more relevant for future surveys, when concurrent carcass prevalence and susceptibility testing could be conducted, as is currently underway in 1999.

The prevalence of *Campylobacter* in chickens was estimated from a 1994-95 survey of **1,297** broiler carcass rinse samples at 88.2% of carcasses, indicating that **1,144** carcasses tested positive (81).

The parameter was thus modeled as:

p_c = Beta(1144+1,1297-1144+1)

4.2 (p_{rc}) - Prevalence of fluoroquinolone-resistant *Campylobacter* among isolates from broiler carcasses

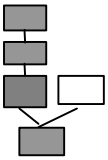
Isolates were collected in a pilot survey by USDA-FSIS, from October to December 1998, from chicken carcass rinse samples (Section 4.1) and cultured as described previously (62). If growth was evident, a single colony was removed from the plate for susceptibility testing. A total of 159 *C. jejuni* isolates were collected from chicken carcasses for the period. The isolates were speciated using the biochemical hippurate assay and polymerase chain reaction (PCR) hippuricase primers to identify hippurase negative *C. jejuni* (55). The proportion of Ciprofloxacin resistant *C. jejuni* isolates was 11.3% (**18**/**159**), and *C.*"*other*" was 20.0% (10/50) (personal communication P. Fedorka-Cray). *C.* "other" were hippurate negative isolates, that were not *jejuni* as identified by PCR for the hippuricase gene and may include species such as *C. lari* that are intrinsically resistant to quinolones.

The level of resistance to Ciprofloxacin in *C. jejuni* from chickens was used in the risk assessment because the greater proportion of human disease, 92.7% in the Campylobacter Case Control Study, was due to *C. jejuni*. *C. coli* were not clearly distinguished from the group *C.* "other" which may have included *C. lari*, a species intrinsically resistant to quinolones, therefore this precluded use of these isolates in the risk assessment.

DISCUSSION: Limitations in determination of the level of fluoroquinolone resistance in *Campylobacter* include: the small number of isolates collected, the lack of seasonal representation and the potential for selection of mixed colonies of organisms when selecting a single colony.

Unquantified Issues in the Assessment of the Prevalence of Resistance in Campylobacter isolates

Other problems were raised with the isolation and susceptibility testing of *Campylobacter*. Lack of agreement of MIC susceptibility test results occurs in up to 10% (2/20) of isolates subjected to repeat testing in one study (personal communication P. Fedorka-Cray). One explanation of the inconsistency is that the single colony may be composed of multiple isolates and that all isolates in the mixed colony may not have the same potential to survive storage, freezing, re-culture and testing. The effect of selecting a colony with multiple isolates on the reliability of sample susceptibility testing is decreased. This effect was not modelled until further data can provide more information about the cause, direction and frequency of the inconsistency.



In addition to the problem mentioned in the paragraph above, many varied *Campylobacter* colonies are present on a culture plate. The selection of a single colony from a plate of diverse colonies provides a "plate average," and when the likelihood of isolating a susceptible isolate is greater than isolating a resistant isolate on a plate, the level may underestimate the true carcass prevalence. Therefore, the level of resistance is not closely linked to carcass numbers or consumption volume. Use of a quinolone-containing screening media would provide a better estimate of the true carcass prevalence and load of Ciprofloxacin resistant *Campylobacter* and may be a more relevant method to use when assessing the impact of resistant pathogens transferred to people on food.

Hippurate positive isolates were tested in one laboratory using *Campylobacter* species specific PCR primers for the *ceu* gene and preliminary findings indicated that approximately 8.2% of hippurate positive animal isolates reacted with *C. coli* specific primers (personal communication, P. Fedorka-Cray). As hippurate negative *C. coli* frequently carry higher levels of resistance to fluoroquinolones, and if this is true for hippurate positive *C. coli*, then the level of resistance within *C. jejuni* may be decreased proportionately. This potential bias has not been identified in another laboratory and since the technique is very sensitive, the information was not considered relevant to the risk assessment until these findings are confirmed in other groups of isolates and validated in other laboratories.

The three issues described above; the lack of reliability of testing, the potential underestimate of the level of resistance and the misclassification of Campylobacter species resulting in decreased levels of resistance in *C. jejuni* are issues that are currently not quantifiable. These issues need to be validated and tested to allow a meaningful assessment of their impact on both human and foodborne isolates. This risk assessment determined the measurable risk, limiting the model to those parameters for which data were relevant, valid and available.

DATA GAP: A yearlong representative survey of broiler chicken carcasses to allow the estimation of a yearly prevalence of *Campylobacter* and the testing of those isolates from broilers for Ciprofloxacin resistance is currently underway with FSIS. These isolates will be tested for susceptibility using the E-Test and data for 1999 should be available in mid-2000.

The parameter was thus modelled as:

p_{rc} = Beta(18+1,159-18+1)

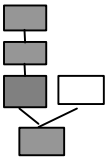
This parameter estimate has a mean of 11.8% and a mode of 11.3% which is very similar to the estimate for p_{rh}, the proportion of *Campylobacter* infections from broilers that are fluoroquinolone resistant, which has a mean of 10.4% and a mode of 9.8%.

4.3 (p_p) - Estimated prevalence of fluoroquinolone-resistant *Campylobacter* in broiler carcasses

This parameter is calculated as:
$p_p = p_c * p_{rc}$

4.4 (c) - Consumption of domestically reared chicken in the United States per head (lbs)
An annual value representing measurable human exposure to chicken in the United States less product sent for rendering, product diverted for pet food, exports, water added during processing and imports was the pounds of boneless broiler food disappearance, which in 1998 preliminary results was 51.4 lbs per capita (79).

c = 51.4 lbs



4.5 (V_c) - Volume of boneless domestically reared chicken consumed by US citizens (lbs)

This parameter is calculated as:

$$V_c = c * n_{US}$$

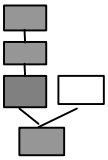
4.6 ($\mathbf{V_i}$) - Total consumption of boneless domestically reared chicken contaminated with fluoroquinolone resistant *Campylobacter* in US (lbs)

This parameter is calculated as:

$$p_p = p_c * p_{rc}$$

It represents the amount of boneless product contaminated with fluoroquinolone resistant Campylobacter consumed in the U.S. in the year. In this model, this quantity is a surrogate for the Poisson intensity with which a US citizen may contract campylobacteriosis from consuming chicken for which fluoroquinolone then prescribed is ineffective. Figure 4.1 shows the uncertainty distribution for $\mathbf{V_i}$.

Model output	5 percentile	Mean	95 percentile
$\mathbf{V_i}$	9.67E+8	1.45E+9	1.99E+9



Section 4 Summary

The mean estimated value for pounds of boneless chicken carrying fluoroquinolone resistant Campylobacter consumed in 1998 is 1,450,000,000 pounds. The 90% confidence interval for the mean is (967,000,000 to 1,990,000,000). Relative contributions of the various components of the model to the model uncertainty will be presented in Section 5, Sensitivity Analysis.

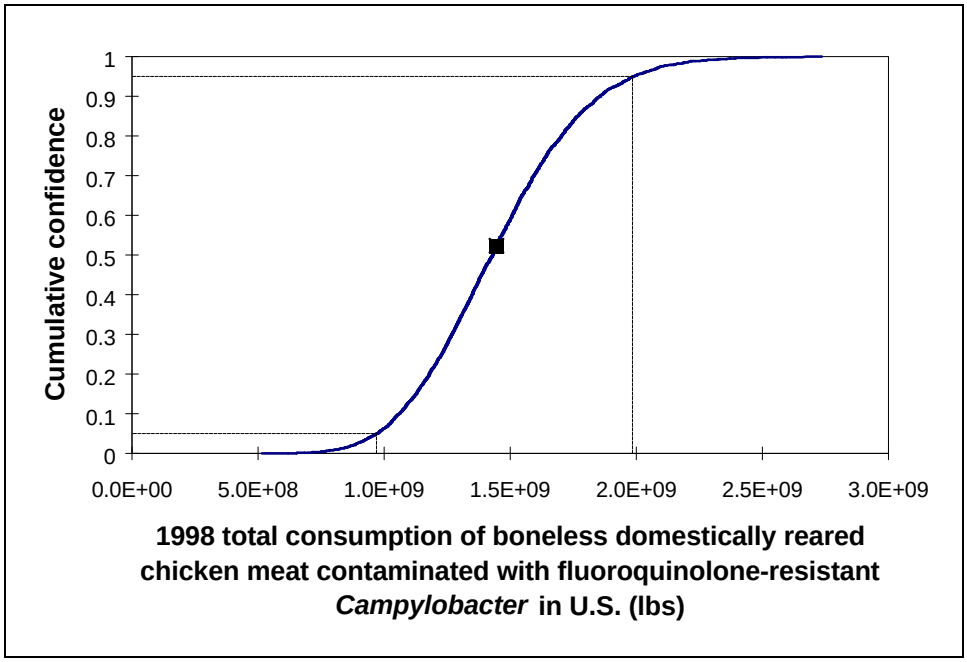


Figure 4.1. The cumulative confidence distribution for $\mathbf{V_i}$.